# DISTRIBUTION OF INHIBITORY QUEEN PHEROMONE AMONG VIRGIN QUEENS OF AN ANT, *SOLENOPSIS INVICTA*

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## Introduction

While they remain in the parental nest prior to mating flights, sexually mature virgin queens of the fire ant, *Solenopsis invicta* Buren, are prevented from shedding their wings and becoming reproductively active by means of an inhibitory primer pheromone produced by the mother queen of the colony (Fletcher and Blum 1981a; 1981b; 1983). This pheromone is relatively nonvolatile and is transferred to virgin queens through physical contacts between colony members (Fletcher and Blum 1981b). When removed from the source of pheromone by separation from the mother queen, some virgin queens dealate in as little as 12 h (Fletcher and Blum 1981b). Hence, inhibitory signals must be transferred frequently to each virgin queen.

The production and transfer of inhibitory signals by the queen honeybee, *Apis mellifera,* illustrates the complexity of queen–worker communication. In 1954, Butler hypothesized that the inhibitory pheromone of the queen honeybee is distributed through food exchange among the workers. A second hypothesis, by which workers contacting a queen pick up queen substance on their bodies and then function as "substitute queens", was proposed by Verheijen-Voogd (1959). Although neither hypothesis has been disproved, most of the evidence supports surface transport as the primary mechanism of pheromone transfer (Velthuis 1972; Butler 1974). Recently, Seeley (1979) showed that physical contacts between the workers and a relatively small number of "messenger

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bees" that have been in contact with the queen during the previous 30 min are sufficient to distribute the pheromonal signal effectively.

Using the studies on honeybees as a model, first we investigated the rate at which radiolabel was transferred to virgin queens from a) food; b) the body surfaces of the mated queen, virgin queens, and workers; and c) small glass rods. The frequency of food transfer between workers and virgin queens in this experiment indicated that feeding might be involved in pheromone transfer. In a second experiment we examined whether feeding activity would increase the quantity of radioactive tracer transferred from the surface of the mother queen to the virgin queens.

## Materials and Methods

*Rate of transfer of food and surface materials.*

A total of 21 monogynous colonies of *S. invicta* were obtained from the field at College Station, Texas and their composition was standardized so that each consisted of about 20,000 workers, 40 cm3 of worker brood, a moderately physogastric mated queen, and 400 sexually mature virgin queens. These colonies were fed on a diet of insects and 50/50 (wt/wt) honey solution and kept at a temperature of 26°C. Colonies were housed in 2 artificial nests made of plaster of paris inside large plastic boxes. A bridge connected the nest box to a foraging arena (Mirenda and Vinson 1982). Colonies were fed continuously before and during testing.

To determine whether food transfer between workers and virgin queens occurred in substantially less than the 12 h required for the first virgins to dealate after orphaning, egg albumin and egg yolk powder were labeled with 125 Iodine (Sorensen and Vinson 1981) and offered singly to foragers along with the normal food supply. Beginning 15 min after the first forager re-entered the nest after leaving this food source, random samples of 10 virgin queens were taken every 15 min, alternating between the two nests for a total period of 2 h. Radiation was measured individually using a Searle 1195 gamma radiation counter. The threshold criterion for the presence of radiolabel was any level of radiation above that of the background radiation plus two standard deviations. Disturbance to the colony was reduced by wearing face masks during sampling to eliminate exposure of the colony to breath $CO_2$ (Wilson 1971) and

by returning the cover of the nest as soon as possible. Each treatment was repeated three times, using a total of six colonies.

Secondly, the rate at which radiolabeled albumin was distributed to virgin queens from the body surface of the mated queen was determined. The mated queen was dipped in $^{125}$I-albumin mixed in phosphate buffer, taking care to avoid contamination of her mouthparts, and allowed to dry for 10 min before returning her to the colony. Samples of virgin queens were taken as before. Roughly 1 picogram ($10^{-12}$gm) of radiolabeled protein adhered to each queen, a quantity not detectable by workers (Sorensen and Vinson 1981). For comparison, a virgin queen, freshly freeze-killed in dry ice with her wings removed, was similarly dipped to determine the rate of distribution of surface materials among virgin queens. Virgin queens inhibited by the mother queen do not themselves produce the inhibitory pheromone (D. J. C. Fletcher, unpublished data). To correlate grooming with transfer of radioactivity, we also treated and tested freshly killed mother queens, two dead major workers together, and a siliconized glass rod (approximately the same surface area of the queen). Killing prevented these from distributing the radiolabel themselves through contact with other workers. Each treatment was repeated three times using a different source colony each time, a total of 15 colonies. Results were analyzed by comparing slopes of the cumulative number of virgin queens labeled vs. time (Newman-Keuls test) (Zar 1974). Quantities of egg yolk and albumin found in virgin queens were compared using the Kruskal-Wallis ANOVA and Post hoc test.

*The role of food in pheromone transfer.*

If virgin queens were fed rapidly and frequently enough, the inhibitory pheromone could be passed to them by contaminated workers during feeding. We examined this possibility by dividing small plastic nests in half using a wire screen which prevented passage of ants from one side to the other, thereby limiting contact to trophallaxis and antennal contact (Fletcher and Blum 1981b). After withholding food from the source colony for 24 h, 74 workers, 35 larvae, 15 virgin queens, and the mated queen were removed and the queen together with 37 workers and the larvae placed on one side of the screen, the virgin queens and remaining workers on the other. The mated queen was then removed, dipped in $^{125}$I-albumin and

returned. After 30 minutes, the radioactivity in each virgin queen was measured (Trial A). The queen was redipped and placed in a clean divided box with new workers, virgin queens, and larvae from the same source colony. Food (*Heliothis* pupae) was added to the queenright side (Trial B) and the experiment repeated. Subsequent trials were conducted with food on the queenless side (Trial C), and, after removing the workers tending the virgin queens, placing food with the virgin queens (Trial D) and placing food on the queenright side (Trial E). These trials were repeated using five other source colonies for a total of 6 replications. Results were analyzed using Scheffe's test to compare mean cpm (counts per minute) per virgin queen.

Each mother queen was weighed as a measure of her degree of ovary development and the quantity of radiolabel removed after each trial determined. Weight loss in fire ant queens is correlated with loss of fecundity and diminished production of inhibitory primer pheromone (Fletcher and Blum 1983). Thus, the weight of our queens could affect the quantity of radiolabel removed and the number of virgin queens labeled.

## Results

*Rate of transfer of food and surface materials.*

Using radiolabeled protein we determined how rapidly radioactivity was distributed from various sources to the virgin queens. When radiolabeled egg yolk or egg albumin was placed in the foraging chamber, 60–100% of the alates sampled contained radiolabel after 15 min. During the first two trials, egg yolk was fed more rapidly to virgin queens than was albumin ($F_{1,4} = 217.3$, $p < 0.001$)(Figs. 1, 2). In addition, the amount of radiolabel retained indicated that virgin queens received 5–30 times more egg yolk by weight than albumin, reinforcing our observations that albumin was not brought into the nest as readily as egg yolk (data not shown). The location of the mated queen in the nest with respect to the virgin queens tested did not significantly affect the distribution of food. During the third trial (C) we noticed that some of the virgin queens in our nests were dealating, indicating that they were no longer receiving adequate levels of pheromone from the mated queen. This was possibly due to weight losses sustained by the queens of 20–30% before beginning the last trial (Fletcher and Blum 1981b). During

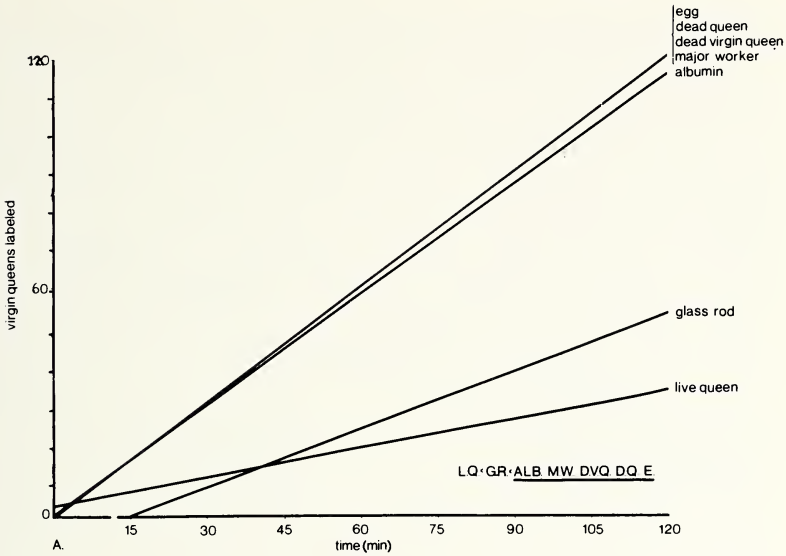


Figure 1. Linear regression analyses on the cumulative number of virgin queens receiving radiolabel during the first trial (A) after the introduction of radiolabeled egg yolk (E) or radiolabeled egg albumin (alb), the reintroduction of the live mated queen (LQ) or freshly killed mated queen (DQ) surface labeled with radiolabel or the introduction of a glass rod (GR), 2 dead major workers (MW), or a dead dewinged virgin queen (DVQ) surface labeled with radiolabel. A total of 10 virgin queens were sampled in each colony at 15 min intervals for a total of 120 virgin queens over the 2 h period. Slopes of the underlined groups do not differ at $p < 0.05$ (Newman-Keuls Test).

these trials the quantity of food fed to the virgin queens dropped significantly ($p < .05$) although the majority were still fed (data not shown).

Virgin queens became radiolabeled more rapidly from the surface of dead queens than from the surface of live queens in two of the three trials (Figs. 1, 2). In the third trial, significantly more virgin queens received radiolabel from the surface of the live queen than from the dead queen (Fig. 3). Virgin queens in the same nest as the queen did not receive significantly more radioactivity than virgin queens in the second nest (data not shown).

There was marked variation in the rate at which radiolabel was transferred to virgin queens from the surface of a small glass rod, 2

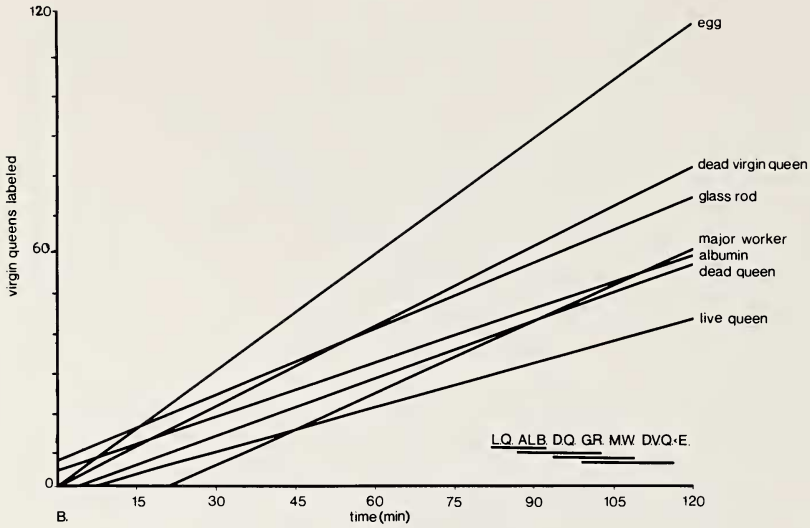


Figure 2. Second trial (B). See Figure 1 for details.

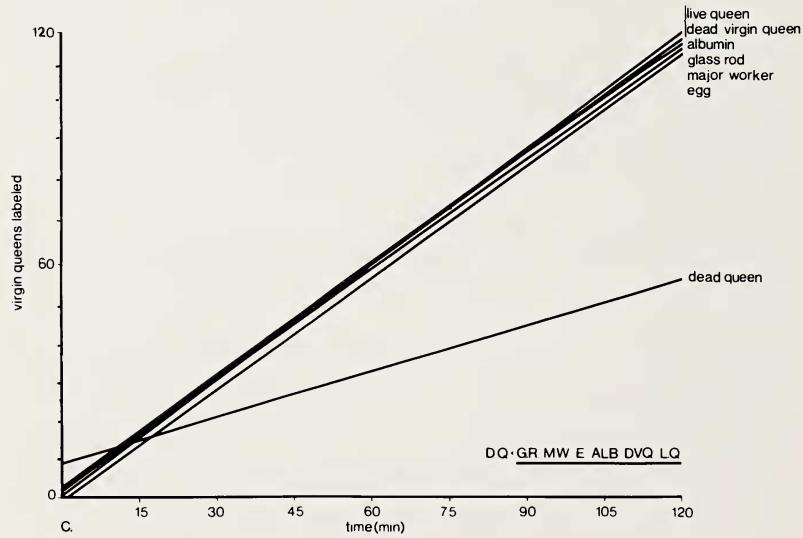


Figure 3. Third trial (C). See Figure 1 for details.

dead major workers, and a dead dewinged virgin queen (Figs. 1,2,3). In all three trials, more virgin queens received radiolabel from the surface of the dead dewinged virgin queen than from the glass rod ($F_{2,18} = 79.3$, $p < 0.001$). In two of the trials, virgin queens received slightly more radiolabel from the glass rod than from the surface of the dead major workers (Figs. 2,3) but in one (Fig. 1), significantly fewer virgin queens received radiolabel from the glass rod than from the major workers. The location of the virgin queens in the nests with respect to the glass rods or dead dewinged virgin queens did not affect the rate of distribution of radiolabel. Significantly more virgin queens from the nest with the queen and treated major workers contained radioactivity than those taken from the second nest ($F_{1,4} = 15.1$, $p < 0.05$).

Combining the results of the three trials, within 120 min 98% of the virgin queens sampled received radiolabeled egg yolk; 87% received radioactivity from the surface of dead dewinged virgin queens; 82% received radioactivity from the surface of dead major workers; 79% received radiolabeled albumin; 68% received radioactivity from the surface of the glass rod; 65% received radioactivity from the surface of a dead mated queen; and 53% received radioactivity from the surface of a live queen. All were significantly different ($F_{6,28} = 127.6$, $p = 0.001$) except for the number of virgin queens receiving radioactivity from either a) radiolabeled albumin or dead major workers and b) glass rods or dead queens.

*Role of food in pheromone transfer.*

Since 98% of the virgin queens sampled were fed radiolabeled egg yolk within 2 h, we examined the possibility that the presence of food might increase the quantity of radioactivity transferred from the surface of the queen to the virgin queens by encouraging food transfer and subsequent surface contacts among workers and queens. The results are given in Figure 4 as the mean number of cpm per virgin queen. Virgin queens tended by workers were separated from the queen, workers, and brood by a fine screen that permitted trophallaxis but restricted grooming. Only the mated queen was surface labeled and the distribution of radioactive tracer from 1 side to the other was dependent on trophallaxis or antennal contacts through the wire mesh. The virgin queens in Trial C, where food was

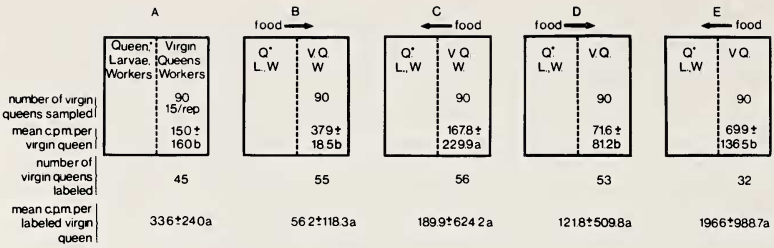


Figure 4. Number of virgin queens containing radioactivity following reintroduction of the mated queen who was surface labeled with radiolabel (*). Five trials (A-E) were conducted on each of 6 colonies with no food, food on the queenright or queenless side, and virgin queens with or without workers. Boxes were divided with screens which allowed trophallaxis and limited antennal contact. Radioactivity (counts per minute or cpm) was measured after 1 h. Results are given as means and standard deviations for all virgin queens measured (within boxes) and for only those virgin queens that received radioactivity (below boxes). Means followed by the same letter in each row are not significantly different at $p < 0.05$ using Scheffe's Test.

placed on the queenless side, received significantly more label than the virgin queens in all other trials (Scheffe's test). Proteinaceous food is normally directed toward the queen and brood (Sorensen and Vinson 1981) so Trial C closely approximated normal food flow conditions. Fewer virgin queens received radioactive tracer (and less of it) when food was absent than when food was present but differences were not statistically significant. Untended virgin queens received radioactivity indicating that workers on the queenright side contacted them through the screen (Trial E) and, when food was placed near them, untended virgin queens contacted workers on the queenright side (Trial D).

The weights of the mated queens used ranged from 16.8 mg to 19.6 mg, considered marginally physogastric by Fletcher and Blum (1983). There was a strong correlation between the weight of the queen and the number of virgin queens receiving radioactivity ($y = 15.71x-257$, $r_2 = 0.67$). The mean percentage of radiolabel remaining on the queen after each trial ranged from 21–39%. This contrasts with a mean of roughly 6% left in the first experiments. The weight of the queen was also strongly correlated with the quantity of radiolabel removed ($y = -0.05x + 1.26$, $r_2 = 0.65$). As the queens decreased in weight, less radiolabel was groomed from their surface and the number of virgin queens receiving radiolabel decreased.

## Discussion

The transfer of food and surface materials to virgin queens in fire ant nests was very rapid. This indicated that rapid transmission of signals or pheromones throughout a colony via trophallaxis, physical contact, and/or mutual grooming was possible. Proteinaceous foods reached the virgin queens within 15 min and up to 98% had received egg yolk after 2 h, well within the 12 h period after which pheromonally disinhibited virgin queens begin to dealate (Fletcher and Blum 1981b). This was expected. In polygynous colonies with 400 workers, queens, brood, and no reproductives, egg yolk is distributed to all of the workers and 80% of the larvae within 10 min (Sorensen et al. 1981) and, in colonies with 4,000 workers, to 95% of the workers and 85% of the larvae within 1 h (Sorensen and Vinson 1981). Results from our third trial indicated that virgin females received significantly less food in colonies when the mated queen was no longer producing enough pheromone to prevent dealation. This suggests that decreased food transfer could have minimized the transfer of pheromone and led to dealation. However, this decrease in feeding could also have been caused by the disruption in normal food distribution as workers began to execute dealates.

The high percentage (68–87%) of virgin queens receiving radioactivity from the surface of dead virgin queens, dead major workers, and glass rods showed that grooming activities and physical contacts between workers and virgin queens were extensive. Mirenda and Vinson (1981) found that active *S. invicta* workers spend 78% of their time grooming themselves, other ants, or being groomed. However, since fire ant workers respond to fresh corpses with vigorous and extended inspection (Blum 1970; Howard and Tschinkel 1976) an initial burst of grooming and inspection by many workers followed by seizure and disposal of the corpses could have been responsible for spreading radioactivity rapidly through the colony. More virgin queens received radioactivity from the virgin queen corpses than from the major worker corpses, although the difference was not statistically significant. Virgin queen corpses may produce competing odors that mask necrophoric signals, delaying their removal and increasing worker–queen corpse contact. The extent of transfer of radioactivity from the virgin queen corpses may indicate that live virgin queens are also groomed and contacted frequently by workers. Workers exhibited aggressive behavior

towards the glass rods and large numbers of workers came into contact with them. The high number of radiolabeled virgin queens resulting from all of these encounters showed that contaminated workers either fed, groomed, or contacted them frequently. This rate of distribution of radioactivity suggested that there was a very high level of contact between colony members. This indicates that all pheromones could be rapidly distributed regardless of their origin. This is of considerable general interest and supports hypotheses of pheromonal control of behavior in social insects (Wilson 1971).

Radiolabel was removed from both the dead mated queens and live mated queens and transmitted to over 50% of the virgin queens within 2 h. Radiolabel was transmitted to more virgin queens from mated queen corpses than from live queens in two of the three trials, possibly because a greater number of workers have access to a dead queen. Seeley (1979) has shown that honeybee workers collect queen substance most heavily when the queen is stationary. Since the live queens used in our study were disturbed frequently by sampling, periods in which they remained stationary were limited. This could have reduced the number of worker–queen contacts and thus the quantity of radiolabel removed. Although radiolabel from the surface of live mated queens was transmitted to virgin queens at a slower rate, its transfer was still rapid enough to insure that it would have reached all of the virgin queens in less than 12 h.

Examining the effect of food distribution on the transfer of radioactivity from live queens to virgin queens, we found that the presence of food increased the quantity of radiolabel transferred. Although not conclusive, our results suggested that food exchange may have been involved in signal transfer. We realise that these results could be misleading. A similar experiment with well fed queenless honeybee workers on 1 side of a wire mesh screen and starved queenright workers on the other side also showed that the queenright group passes "information" to the queenless group during transfer of food but that food and information travel in opposite directions (Verheijen-Voogd 1959). Also, recent research supports surface transport over food exchange as the primary mechanism of honeybee queen substance transmission (Seeley 1979). On the other hand, food exchange between workers has been implicated in the transfer of pheromone produced by immature honeybee queens (Free and Ferguson 1982). Because the frequency and extent of food

transfer to virgin fire ant queens is high, we cannot rule out food exchange as the primary mechanism for the transfer of inhibitory queen pheromone in fire ant colonies.

Our results from the second experiment also agree with the hypothesis that the quantity of inhibitory pheromone produced is positively correlated with fecundity of the mother queen (Fletcher and Blum, 1983). The mated queens used in this study were only slightly physogastric, thus evidently limiting both the amount of inhibitory pheromone produced and their attractiveness to workers. As predicted, the quantity of radioactivity removed and transferred to virgin queens decreased as the weight of the mated queens decreased.

## Summary

Two ways in which an inhibitory queen pheromone of the ant, *Solenopsis invicta* Buren might be distributed by workers to virgin queens in a colony, trophallaxis and surface transport, were investigated.

Using a nonvolatile radiolabeled protein in quantities nondetectable to the ants, evidence suggested that physical contact was an efficient mode of transmission for inhibitory pheromone. On the other hand, when radiolabeled material was incorporated into food, virgin queens quickly received it. A second study indicated that the transfer of food between workers and virgin queens increased the amount of surface contact and grooming and thus could aid in the distribution of inhibitory pheromone. This suggests that trophallaxis may be an additional means of distributing queen pheromones in this species.

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